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Progression in Osteoblastic Bone Metastases

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14. ABSTRACT In this project, we are studying the role of D32G-mutant beta-catenin in the expression of secretory genes by prostate cancer cells. We believe that D32G-mutant beta-catenin potentially activates a subset of beta-catenin/wnt downstream target genes, thus providing a tool for identifying "bone progression" factors activated by this pathway in prostate cancer. Results from the studies performed during this period indicate that activation of beta-catenin in prostate cancer cells stimulates a subset of beta-catenin target genes and suggest that beta-catenin expression in prostate cancer cells mediates the prostate cancer-induced new bone formation in vitro and in vivo. These results provide confidence that our gene-expression studies will be informative for identifying the beta-catenin downstream target genes that mediate the osteoblastic phenotype induced by prostate cancer cells. Immunohistochemical studies performed in human bone metastases of prostate cancer identified 4 groups based on beta-catenin intracellular distribution and expression and androgen receptor expression. It will be interesting to assess how the selected factors induced by beta-catenin in prostate cancer cells are expressed in the different groups. Although these results would only be correlative, they would provide the basis for prioritization in future studies.					
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PROGRESS REPORT: Beta-catenin/TCF signaling and castrate-resistant progression of osteoblastic bone metastases

INTRODUCTION: In this project, we are studying the role of D32G mutant beta-catenin in the expression of secretory genes in prostate cancer cells. We believe that D32G-mutant beta-catenin potently activates a subset of beta-catenin/wnt downstream target genes, thus providing a tool for identifying secretory genes in prostate cancer that mediate the prostate cancer-induced osteoblast proliferation and new bone formation. . The overall goal is to understand how the prostate cancer-bone interaction affects prostate cancer growth in bone during human prostate cancer bone metastasis.

BODY

Task 1. Determine whether expressing the beta-catenin mutant D32G in prostate cancer cells affects their ability to induce osteoblast activation *in vitro* and whether these prostate cancer cells grow more robustly in the presence of these activated osteoblasts.

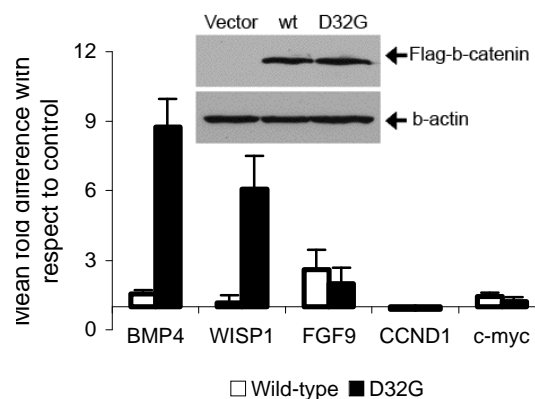
- a Using PC3 and DU145 prostate cancer cells, develop stably transfected clones expressing empty vector, wild-type beta-catenin, or the D32G mutant beta-catenin.
 - Select clones with high and low TCF transcriptional activity (10 and 50 times above transcriptional activity levels using reporter plasmid containing mutant TCF binding sites).
 - Confirm myc–beta-catenin expression and nuclear localization in clones by Western blotting and confocal microscopy (using anti-myc tag antibody).

We first performed transient transfections of prostate cancer cells to establish the feasibility of expressing beta-catenin in these cells. Also, because the main objective of overexpressing beta-catenin in prostate cancer cells was to recapitulate our findings with the osteogenic prostate cancer cell MDA PCa 118b, we assessed whether beta-catenin induces the expression of known downstream target genes found in MDA PCa 118b cells.

We used expression vectors expressing either wild-type or D32G-mutant beta-catenin under the control of a cytomegalovirus promoter in which each polypeptide was expressed as fusion with a 3xFlag epitope tag (Sigma-Aldrich). The D32G mutant was generated by nucleotide substitution of wild-type beta-catenin with the use of a QuikChange site-directed mutagenesis kit (Stratagene). The mutant was confirmed by direct sequencing. We then transiently transfected empty vector, wild-type, and D32G-mutant beta-catenin separately into PC3 cells. The cells were used to isolate cell extracts and total RNA to measure the expression of beta-catenin by Western blotting and its various target genes by real-time RT-PCR. Western blotting using anti-Flag antibody showed that the 2 polypeptides were expressed in PC3 cells at an almost equal level (Fig. 1, inset). We thought it interesting that gene-expression analysis demonstrated that D32G-mutant beta-catenin strongly stimulated increased expression of BMP4 and WISP1 by factors of 8 and 6, respectively (Fig. 1). Wild-type beta-catenin also induced BMP4 and WISP1, but at lower levels (Fig. 1). This also showed that both D32G and wild type modestly stimulated expression of FGF9. In contrast, neither the D32G mutant nor the wild type significantly changed the expression of c-myc, SOX2, or cyclin D1. This analysis indicated that beta-catenin specifically stimulated expression of the *BMP4* and *WISP1* genes, 2 downstream target genes in the beta-catenin–TCF pathway. Stronger activation of the target genes by D32G-mutant beta-catenin indicated that the D32G mutant is likely more localized in the nuclei of PC3 cells and thus acted as a potent transcriptional activator.

In summary, the results of these studies suggest that activation of beta-catenin in prostate cancer cells stimulates a subset of beta-catenin target genes.

Fig. 1. Relative mRNA level of various genes in PC3 cells 48 hours after transient expression of wild-type (wt) or D32G-mutant beta-catenin. mRNA levels are expressed relative to those in cells transfected with vector control. Total RNA was isolated with an RNeasy mini kit (Qiagen) from PC3 cells 48 hours after transient transfection of each expression construct. The relative mRNA level for each gene was quantified by using real-time RT-PCR with SYBR Green (Applied Biosystems). mRNA levels for each gene were calculated from the values in the linear range of PCR cycles and were normalized with the values of the control mRNA of *GAPDH*. For each gene, data from 3 independent reactions were used to calculate means and standard deviations. The degrees of the differences were calculated from the mean values of each set. **Inset.** Western blot analysis of lysates from PC3 cells transiently transfected with empty vector, wt, or D32G-mutant beta-catenin using anti-Flag antibody or beta-actin.



As presented in our preliminary studies, comparative gene expression analysis between MDA PCa 118, PC3, and MDA PCa 2b cells showed increased expression of several genes that are downstream targets of beta-catenin/TCF, the canonical wnt pathway. We then confirmed these findings by using quantitative gene-expression analysis with real-time RT-PCR. In this case, cDNA was also prepared from prostate cancer xenografts grown subcutaneously in male SCID mice. For this analysis, we also used xenografts of the PC3 prostate cancer cell line because it is bone derived and does not express androgen receptor (AR), although it is osteolytic when injected into the bone of immunodeficient mice. We found that expression of FGF9, BMP4, ITF2, WISP1, and SOX2 was increased by factors of 287, 67, 60, 440, and 133 times, respectively, in MDA PCa 118 vs. PC3 xenografts (Table 1). Expression patterns of these genes were similar in the MDA PCa 118 vs. MDA PCa 2b xenografts (Table 1). Expression of the control gene, *OCT4*, was not significantly different in these 3 xenografts. Finally, AR was highly expressed only in the MDA PCa 2b cells, not in the MDA PCa 118 or PC3 xenografts. *Collectively, these findings further suggest that beta-catenin–wnt signaling is up-regulated in MDA PCa 118 cells.*

Table 1. Relative mRNA level ($\times 10^{-4}$) of various genes in different tumors in comparison to *GAPDH*^a

Gene	Relative level of mRNAs in subcutaneous tumors		
	MDA PCa 118	PC3	MDA PCa 2b
<i>FGF9</i>	863 ± 121 ↑	3 ± 0.2	Undetectable
<i>BMP4</i>	1331 ± 190 ↑	19 ± 4	Undetectable
<i>ITF2</i>	2783 ± 197 ↑	46 ± 6	Undetectable
<i>WISP1</i>	1771 ± 238 ↑	4 ± 1	5 ± 2
<i>SOX2</i>	7904 ± 121 ↑	59 ± 10	24 ± 2
<i>OCT4</i>	66 ± 6	62 ± 11	158 ± 31
<i>AR</i>	Undetectable	7 ± 2	2870 ± 820
<i>Cyclin D1</i>	63 ± 27	505 ± 193	823 ± 115
<i>c-myc</i>	424 ± 57	1500 ± 479	1552 ± 336

^aTotal RNA was isolated from each tumor sample with an RNeasy mini kit (Qiagen). The relative mRNA level for each human gene was quantified with real-time RT-PCR with SYBR Green (Applied Biosystems). mRNA levels for each gene were calculated from the values in the linear range of PCR cycles and was normalized with the values of control mRNA of human *GAPDH*. For each gene, data from 3 independent reactions were used to calculate means and standard deviations. If no value in the linear range of PCR cycles of a specific RNA was detected, it was labeled as undetectable, probably because little or no mRNA was expressed in that particular tumor sample. Primers were human specific, and these were tested using mRNA isolated from PMOs and PC3 cells grown *in vitro*. The vertical arrows indicate the genes that are up-regulated in MDA PCa 118 compared with those in both PC3 and MDA PCa 2b cells.

We found it interesting that our analysis also showed that 2 beta-catenin–TCF target genes, *cyclin D1* and *c-myc* [1], were not induced in MDA PCa 118 cells; instead, expression of these 2 genes was significantly lower in MDA PCa 118 cells than in both PC3 and MDA PCa 2b cells (Table 1). These results are in accord with our findings in PC3 overexpressing beta-catenin (Fig. 1) and suggest that this model recapitulates findings in MDA PCa 118 cells.

We then proceeded to establish stable clones with these PC3 cultures. However, we found that PC3 cells do not survive long term after transfection with beta-catenin. However, we were able to establish stable clones of PC3 expressing empty vector. Similar problems were encountered with DU145 cells. When we tried to use early passages of selection, we could not detect beta-catenin overexpression as compared to controls. We reasoned that overexpression of beta-catenin may need a specific genetic background to be stably incorporated into prostate cancer cells. Thus, we went to Task 2a to silence beta-catenin in MDA PCa 118 cells so that we could use MDA PCa 118 cells with silenced beta-catenin and controls to perform Task 2.b and to study the interaction between prostate cancer cells with silenced beta-catenin and controls and primary mouse osteoblasts *in vitro* (Task 1.b).

Task 2. Determine the effect of expressing the D32G beta-catenin mutant in prostate cancer on the growth of those cells in bone and the bone's reaction to the presence of those cells in an androgen-depleted environment *in vivo*. For these experiments, we used the MDA PCa 118 cells with silenced beta-catenin.

- a. Silence beta-catenin in MDA PCa 118 cells by infecting primary cultures of MDA PCa 118 xenografts with lentivirus vectors expressing shRNAs for beta-catenin (Mission RNAi; Sigma Aldrich); non-targeted shRNA transduction particles will be used as controls.

To determine whether beta-catenin–TCF pathway signaling in MDA PCa 118 prostate cancer cells has any effect on prostate cancer–induced paracrine activation of osteoblasts, we cocultured PMOs and MDA PCa 118b cells with and without knockdown of beta-catenin. This knockdown in MDA PCa 118 cells was done using siRNA, transiently transfected into MDA PCa 118 cells using a Nucleofector kit (Amaxa/Lonza). In this experiment, MDA PCa 118 cells were first transiently transfected with either beta-catenin–specific siRNA (si-bcat) or non-targeted siRNA (si-control). At 24 hours after the transfection, the cells were cocultured with PMOs (primary mouse osteoblasts) so that the 2 cell lines were separated by inserts; thus the cells were sharing medium but were not in physical contact. At 48 hours after coculturing, osteoblast numbers were counted, and the beta-catenin level in MDA PCa 118 cells was measured by using quantitative RT-PCR (Fig. 2, left). This showed that a significant increase of PMO proliferation, with 2-fold increase in cell numbers, occurred after coculturing with MDA PCa 118b cells without beta-catenin knockdown (PMO co si-control-118b) (Fig. 2). In contrast, when coculturing was performed with MDA PCa 118 cells *with* beta-catenin knockdown (PMO co si-bcat-118b), the increase in PMO cell proliferation was less significant, with less than a 1.5-fold increase in cell numbers. Under this coculture condition, the number of MDA PCa 118 cells was mostly unchanged, and thus PMOs were cocultured in the presence of the same number of si-control-118b or si-bcat-118 cells. This then supports the idea that beta-catenin knockdown in MDA PCa 118 cells reduces the level of paracrine factor(s), resulting in a decreased level of stimulation in PMO proliferation and suggesting that beta-catenin–TCF signaling in MDA PCa 118 cells controls production of paracrine factor(s) that could stimulate PMO proliferation.

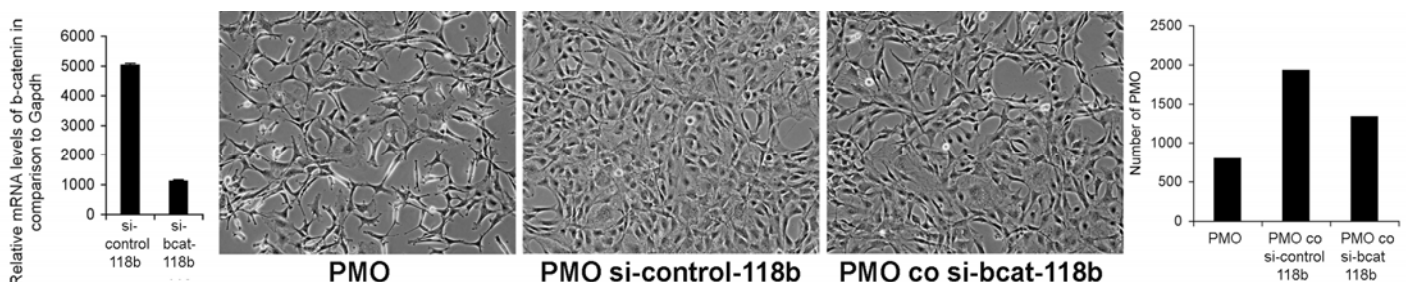


Fig. 2. Left. The relative beta-catenin mRNA level was quantified by real-time RT-PCR in MDA PCa 118b cells transfected with either beta-catenin–specific siRNA (si-bcat) or non-targeted siRNA (si-control). mRNA levels were

calculated from the values in the linear range of PCR cycles and were normalized with the values of control mRNA of *GAPDH* in a manner similar to that described in Table 1. **Middle.** Photomicrograph of PMOs grown alone (PMO), in coculture with si-control transfected MDA PCa 118b (PMO co si-control-118b), and in coculture with si-bcat transfected MDA PCa 118b (PMO co si-bcat-118b). **Right.** PMOs shown in the left panel were counted using a hemocytometer, and results were plotted. Similar results were obtained in 3 independent experiments.

These results demonstrate the efficiency of beta-catenin silencing and confirm that beta-catenin mediates the MDA PCa 118–induced osteoblast activation.

Task 2.b. Inject MDA PCa 118 controls and cells with silenced beta-catenin into the femur of SCID mice.

In Task 2.a., we used 2 different methods to inhibit expression of beta-catenin in MDA PCa 118 cells by knocking down expression of the *beta-catenin* gene: an siRNA (s438; Applied Biosystems/Ambion) and an shRNA (sh1-bcat) expressing lentiviral particles (TRCN0000003845; Sigma-Aldrich). That study showed that knockdown of beta-catenin with either siRNA or shRNA resulted in decreased expression of BMP4 and WISP1. We also previously validated beta-catenin knockdown using another shRNA (sh2-bcat) expressing lentiviral particles (TRCN0000003844; Sigma-Aldrich). We thus used these 2 lentiviral particles to knock down beta-catenin in MDA PCa 118 cells so we could develop stable clones. As a control, a non-targeting shRNA (sh-control) expressing lentiviral particles was used. However, we could not establish stable clones. Part of the difficulty is that MDA PCa 118 cells cannot sustain long term *in vitro* growth. We also tried to infect the cells with lentivirus particles and directly inject them into mice, but the efficiency of infection was about 10%, so the step of *in vitro* selection for enrichment of lentivirus-expressing cells could not be avoided. Thus we decided to perform transient transfections with siRNA transfected into MDA PCa 118b cells using a Nucleofector kit (Amaxa/Lonza), which has an efficiency of transfection of about 50%. We injected siRNA-transfected cells into the bones of SCID mice within 24 to 48 hrs of transfections and proceeded to Task 2.c in 4 to 6 weeks after cell injection. Initial attempts at injecting these cells into the femurs of SCID mice did not render tumors. Then we optimized the transfection procedure to increase cell survival; with this optimized method, the injected cells developed tumors after injection into the bones of SCID mice.

Task 2.c. Obtain and analyze x-ray, magnetic resonance, and micro-computed tomographic images and bone histomorphometric measures (bone mass and osteoclast numbers).

Initial analysis of the first successful tumor development after injection of MDA PCa 118 cells transfected with si-bcat or si-control suggested that there is increased bone mass in the bones injected with MDA PCa 118 cells transfected with si-controls but not in those injected with si-bcat-MDA PCa 118 cells (Fig. 3). This could be due to a reduction of tumor size in the si-bcat-MDA PCa 118 cells compared with controls or to a reduction of the prostate cancer–induced new bone formation by the si-bcat-MDA PCa 118 cells. We are now analyzing these tumor-bearing bones by MRI, micro-CT analysis, bone histomorphometry, and histology to quantify tumor volume and bone mass.

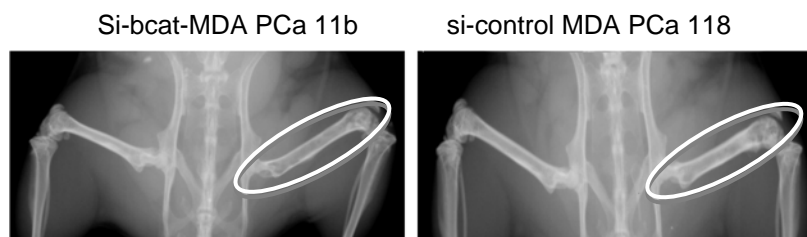


Fig. 3. MDA PCa 118b cells transfected with si-bcat or si-controls growing in the bones of immunodeficient mice. X-rays show mouse pelvis and rear limbs 6 weeks after intrafemoral implantation of MDA PCa 118a cells. Injected femur is circled.

MDA PCa 118 cells used to perform these studies have to be isolated from MDA PCa 118 cells growing subcutaneously in SCID mice because the cells cannot sustain *in vitro* growth. A large number of cells are

required to perform these studies because many cells are lost in each of the several steps involved, namely cell isolation from tumors, transient transfection, and selection of viable cells by cell attachment in cell culture dishes with 24 hours of plating. Thus, a limited number of mice can be injected every time. Consequently, we will repeat this experiment twice more with 3 mice per arm (3 injected with MDA PCa 118 cells transfected with si-bcat and 3 with si-controls).

Task 3. Identify genes induced by the D32G beta-catenin mutant in prostate cancer cells that mediate osteoblast activation and are expressed in human prostate cancer tissue specimens (months 4–36).

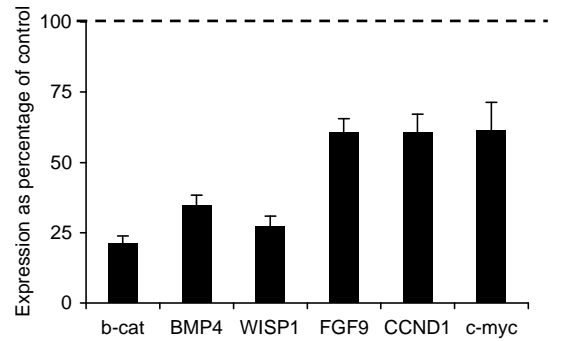
- Identify genes whose expression is regulated by the D32G mutant in prostate cancer cells. Examine the expression of “signature genes” in the beta-catenin/TCF pathway that are activated in MDA PCa 118 xenografts (TCF4, fz d1, NrCAM, CD44, MYCBP, FGF9, FGF18, BMP4, WISP, and RUNX1 genes).
- Perform a gene array analysis by using human GeneChip Arrays (Human Genome U133A microarray; Affymetrix).

We assessed the effect of beta-catenin silencing on the expression of candidate beta-catenin–TCF pathway downstream target genes expressed by MDA PCa 118b cells. For this experiment, MDA PCa 118b cells were isolated from subcutaneous tumors developed in SCID mice. Briefly, tumors were digested with Accumax (Innovative Cell Technologies) for 30 minutes at 37°C. The cell suspensions were filtered through a 70-micron cell strainer, gently loaded onto a Histopaque-1077 gradient, and centrifuged at $400 \times g$ for 30 minutes, and then the upper layer containing mononuclear cells was carefully removed. The cells were washed with phosphate-buffered saline and cultured in CnT52 medium (human prostate epithelium medium from Millipore) in petri dishes or 6-well plates coated with FNC medium. We have observed that MDA PCa 118b cells can be cultured in CnT52 medium for 7–10 days with no change in beta-catenin target gene expression and, more importantly, that these MDA PCa 118b cells cultured for this short term retain their tumorigenic and osteogenic properties when they are injected back either subcutaneously or intrafemorally into SCID mice. Thus, we used these cells to identify beta-catenin target genes after knockdown of beta-catenin mRNA using RNAi.

We used transient transfection of siRNA and lentiviral-mediated shRNA expression to knock down beta-catenin in MDA PCa 118b cells, which were used after 2 days' culturing after their isolation from a tumor. A validated beta-catenin–specific siRNA (s438; Ambion/Applied Biosystems) and, as a control, a non-targeted siRNA were transiently transfected into MDA PCa 118b cells using a Nucleofector kit (Amaxa/Lonza). For shRNA expression, MDA PCa 118b cells were infected with lentivirus particles expressing beta-catenin–specific shRNA (TRCN0000003845; Sigma-Aldrich) and, as a control, with lentivirus particles expressing non-targeted shRNA. At 48 hours after transfection or infection, cells were collected to isolate total RNAs, which were then used to measure expression of genes by quantitative RT-PCR. In this experiment, transient transfection by siRNA reduced the beta-catenin mRNA level by 80%, whereas shRNA expressed by lentiviral vector was reduced by 65%. Fig. 4 shows the results of our analysis of target gene expression after knockdown of beta-catenin by transient transfection of siRNA. This demonstrated that knockdown of beta-catenin significantly reduced the expression of BMP4 and WISP1 by 70% and 75% but modestly reduced (by almost 25%) the expression of FGF9, cyclin D1, and c-myc. This indicated that the expression of BMP4 and WISP1 in MDA PCa 118b cells is highly dependent on beta-catenin–TCF signaling since their expression was also activated in PC3 prostate cancer cells after expression of both wt and D32G-mutant beta-catenin, suggesting that both BMP4 and WISP1 constitute a subset of beta-catenin–TCF target genes that are directly regulated by beta-catenin–TCF signaling in prostate cancer cells.

We subsequently performed a comparative gene array analysis (HuGene 1.0 ST; Affymetrix) between MDA PCa 118 cells with silenced beta-catenin and controls. For this, we used a control group with 4 replicates. For beta-catenin silencing, we used siRNA (s438; Ambion/Applied Biosystems) beta-cat-si1 (4 replicates). We also used second validated siRNA [b-cat-si2 (3 replicates)]. These siRNAs reduced beta-catenin mRNA levels by factors of 6 and 3, respectively. Initial statistical analysis demonstrated that for the mixed linear model, 10 genes were expressed differently between the groups of control vs. beta-ca-si1 at the false discovery rate at 0.05. Initial analysis also demonstrated that the arrays were highly consistent between replicates both within treatments and between treatments. We are now in the process of validating these results.

Fig. 4. Expression of selected genes in MDA PCa 118b cells with silenced beta-catenin. Beta-catenin (b-cat) was silenced in MDA PCa 118b cells by transient transfection with siRNA (s438). As a control, the cells were transfected with non-targeted siRNA. Gene expression was determined by quantitative RT-PCR using total RNAs isolated 48 hours after transfection using a method similar to that described in Fig. 5.



Task 3.a. Study the role of secretory factors identified in Task 1.a. in the osteoblast activation induced by prostate cancer cells.

- Identify secreted factors that are downstream targets of D32G-mutant beta-catenin.
- Silence these factors with lentivirus vectors expressing shRNA (Mission RNAi) and perform the same experiments as described in Task 1.a.

Task 3.b. Study the expression of secretory factors (identified in Task 1.a.) in human prostate cancer tissue specimens (5 normal prostate, 20 primary prostate cancer, and 20 bone metastases from prostate cancer).

- Assess the expression of AR and beta-catenin, and screen for mutations in the coding region of the *beta-catenin* gene.

In the canonical pathway, activation of Wnt signaling stabilizes free cytosolic beta-catenin, which is then translocated to the nucleus [2-4], where it heterodimerizes with transcription factors of the TCF/LEF family and activates the expression of specific genes [2-4]. Accumulation of beta-catenin is therefore critical for activation of the Wnt transcriptional response of the canonical pathway and positive nuclear immunostaining of beta-catenin is an indication of pathway activation. Thus, in preparation to assess the expression of secretory factors in human prostate cancer tissue specimens, we initiated studies of the association between beta-catenin cytoplasmic and/or nuclei or membranous accumulation and AR expression in 10 cases of bone metastases from castrate-resistant human prostate cancers. We performed these immunohistochemical studies in consecutive formalin-fixed paraffin-embedded tissue sections. Cytoplasmic and/or nuclear beta-catenin staining was present in 6 cases, whereas exclusive membranous beta-catenin staining was found in 4 cases (Table 2). Of the 6 cases, 3 had little or no AR expression, indicating that at least 30% of the human prostate cancer bone metastases had activation of beta-catenin–TCF or wnt canonical pathway signaling (groups A and B, Table 2). Further analysis of the beta-catenin and AR immunostaining of these samples subdivided the results into 4 different patterns (Table 2). Of particular interest, 2 samples (including results of donor tissue that generated MDA PCa 118b xenograft) that contained the highest nuclear expression of beta-catenin did not express AR (Group A, Table 2, and Fig. 5). Two other cases had areas with cytoplasmic and/or nuclear beta-catenin staining and heterogeneous or weak AR staining (Group B, Table 2, and Fig. 5). Among 7 cases with strong AR staining, 4 had mostly membranous beta-catenin staining (Group B, Table 2, and Fig. 5), whereas the remaining 3 displayed diffuse beta-catenin staining in the cytoplasm and/or nucleus and membranes (Group D, Table 2).

Finally, evaluation of the bone phenotype in the area from which we took the tissue specimen that we analyzed for the pattern of beta-catenin and AR expression demonstrated that groups A and B displayed an osteoblastic phenotype, thus further supporting our hypothesis (Table 2). We are now in the process of expanding these studies to include more cases as proposed.

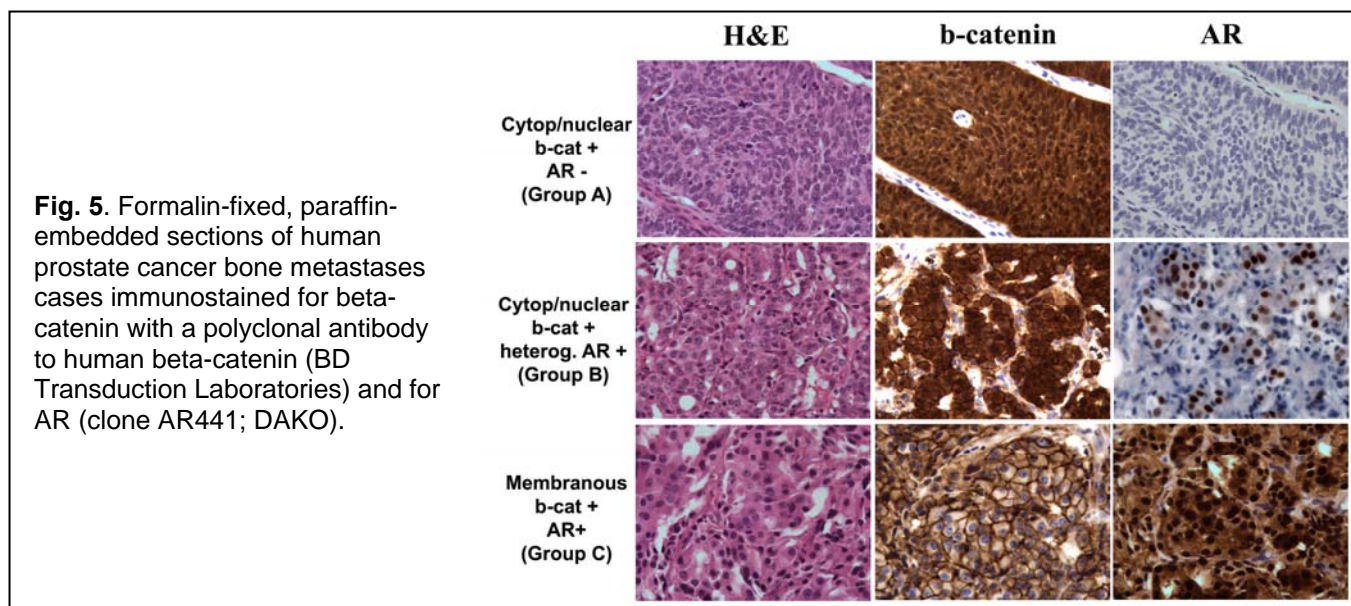
Table 2. Grouping of human prostate cancer bone metastases based on beta-catenin staining and cellular localization and on AR immunostaining of tissue specimens derived from bone metastases^a

Group	Patterns of beta-catenin and AR staining	Beta-catenin cytop/nuclear		Beta-catenin membranous	AR	No. of cases	Bone phenotype ^b		
		Cytop	Nucl				B	M	L
A	Cytop/nuclear b-cat+/AR–	3+/2+	3+	+/–	–	2 ^c	2	0	0
B	Cytop/nuclear b-cat+/heterogeneous AR+	3+/2+	3+/2+	+/–	Heterogeneous	2	2	0	0
C	Cytop/nuclear b-cat–/AR+	–	–	1+/2+/3+	3+/2+	4	2	1	1
D	Cytop/nuclear b-cat+/AR+	3+/2+	2+/–	3+/2+	3+/2+	3	0	2	1

^aAll sections were from formalin-fixed, paraffin-embedded tissue specimens derived from bone metastases. Sections were decalcified in formic acid during fixation -and-embedding process, as previously described [5]. The samples were stained following standard procedures. Slides were read independently by the 2 investigators (NMN and SM) and classified according to staining intensity (0 or 1+ to 3+) and intracellular localization. Evaluations were concordant in 90% of the readings; differences were resolved by consensus after joint review. Cytop, cytoplasmic.

^bBone phenotype was assessed by analysis of x-ray from the site where the tissue specimen that we analyzed was taken: B, osteoblastic; M, mixed osteoblastic and osteolytic; and L, osteolytic.

^cIncludes results of donor tissue that generated the MDA PCa 118b xenograft.



KEY RESEARCH ACCOMPLISHMENTS

- Our study results suggest that activation of beta-catenin in prostate cancer cells stimulates a subset of beta-catenin target genes.
- We demonstrated the efficiency of beta-catenin silencing in MDA PCa 118 cells and confirm that beta-catenin mediates the MDA PCa 118–induced osteoblast activation.
- We successfully obtained tumor development after intrafemoral injection of MDA PCa 118 cells transfected with si-bcat or si-control.
- We successfully performed gene array analysis of beta-catenin silencing in MDA PCa 118 and controls.
- Initial study of beta-catenin intracellular distribution and expression and AR expression in human bone metastases of prostate cancer support the concept that that beta-catenin–TCF signaling mediates the castrate-resistant, osteoblastic progression of prostate cancer in bone independently of AR in a subpopulation of men with prostate cancer bone metastases. This is in alignment with our hypothesis.

REPORTABLE OUTCOMES

Principal Investigator, Beta-Catenin and Prostate Cancer Bone Metastases. R01-CA134769-01, NIH/NCI Priority Score: 186, Resubmitted 3/2009, 10/1/2008–9/30/2013.

Principal Investigator, FGF and Beta-Catenin/Lef1 Signaling in Prostate Cancer Bone Metastases. PC093112—INVITATION TO SUBMIT AN APPLICATION. Fiscal Year 2009 (FY09) Department of Defense (DOD) Prostate Cancer Research Program (PCRP) Idea Development Award.

CONCLUSION

Our *in vitro* and *in vivo* study results support our hypothesis that *beta-catenin–TCF signaling in prostate cancer cells mediates prostate cancer–induced osteoblast activation and new bone formation*. These results provide confidence that our gene-expression studies will be informative and constitute a good tool for identifying beta-catenin downstream target genes that mediate the osteoblastic phenotype induced by prostate cancer cells. Furthermore, our immunohistochemical studies in human bone metastases of prostate cancer (Table 2) identified 4 groups based on beta-catenin intracellular distribution and expression and AR expression. We expect that this segregation of cases will remain when we expand the number of cases studied. If that is the case, it will be of interest to assess how the selected factors induced by beta-catenin in MDA PCa 118 cells (selected in Tasks 1 through 3) are expressed in the different groups. Although these results would only be correlative, they would provide the basis for prioritization in future studies.

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